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(54) Title: DNA EXPRESSING F_c RECEPTOR PROTEIN

(57) Abstract

Substantially pure DNA expressing F_c receptor proteins is taught. Additionally, the proteins thus expressed and applications employing these are set forth as well.

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DNA EXPRESSING F_c RECEPTOR PROTEIN

FIELD OF THE INVENTION

This invention relates to immunoglobulin receptors. In particular, it relates to nucleotide sequences which express the receptor molecules, the receptor molecules themselves, as well as transformed cell lines which produce the molecules. In addition, methods for producing the protein are taught. Finally, also taught are analogs to organs which are associated with immunoglobulin receptors.

BACKGROUND AND PRIOR ART

For the immune system to achieve the function of protecting the organism against foreign antigens, cooperation between the humoral and cellular pathways occurs via interaction of antibody-antigen complexes with effector cells, mediated by specific antibody receptors, known as Fc receptors. These receptor molecules act in a critical way to mediate binding of antibodies to effector cells, as well as in the regulation of antibody function.

Receptors for the Fc domain of immunoglobulin G, which is the most common class of immunoglobulin, are known to be present on B cells, some T cells, natural killer or "NK" cells, macrophages, and polymorphronuclear leukocytes. In

this regard see, e.g. Unkeless, et al., Adv. Immunol. 31: 247-270 (1981); Springer, et al., Contemp. Top. Immunobiol. 13: 1-31 (1984); Dickler, Mol. Immunol. 19: 1301-1306 (1982).

When immune complexes bind to Fc receptors on neutrophils and macrophages, this triggers cellular responses which include phagocytosis, release of activated oxygen metabolites, and mediators of inflammation such as leukotrienes and prostaglandins, as well as induction of neutral hydrolases. See, e.g. Nathan, et al., N. Eng. J. Med. 303: 622 (1980). Fc receptors have been described on lymphocytes as well: Uhen, et al., Cellular Immunol. 95: 368-379 (1985), where they are presumed to have a role in the modulation of antibody production by B cells.

Fc receptors have been described for all classes of immunoglobulin (IgA, IgD, IgE IgG, IgM), but very little is known about the molecules themselves. Perhaps the best characterized receptors are what are known as the "high avidity basophil/mast cell" IgE receptor (FcE), and mouse macrophage Fc receptor which binds IgG2b/IgG1 immune complexes (FcG2b/1R). With respect to murine specimens, studies of competitive binding of different IgG subclasses (Diamond, et al., J. Exp. Med. 150: 721-726 (1979); J. Immunol. 125: 631-633 (1980); J. Exp. Med. 153: 514-519 (1981), and differential sensitivity to proteases (Unkeless, J. Exp.

Med. 142: 1520 (1975); have demonstrated the presence of binding sites for IgG3 (FcG3R), IgG2a (FcG2aR), and IgG2b/G1 complexes (FcG2b/1R). The latter molecule has been described as an integral membrane glycoprotein of 50-60 K daltons, with four sites for N-linked glycosylation. See, in this regard, Green, et al., J. Biol. Chem. 260: 9867-9874 (1985).

Recent work has made it necessary to obtain detailed biochemical characterization of receptors. A monoclonal antibody, 2.4G2 is known which is directed against an epitope present on murine FcG receptors of both macrophages and lymphocytes. Unkeless, J. Exp. Med. 150: 580-596 (1979). While the epitope recognized is known to be present on macrophages and lymphocytes, work by, e.g. Phillips, et al., J. Immunol. 134: 2835-2838 (1985); Baum, et al., J. Exp. Med. 162: 282-296 (1985), and Teilland, et al., J. Immunol. 134: 1774-1779 (1985), shows that the isotype specificity of the lymphocyte receptor active with 2.4G2 is broader than FcG2b/1R, which does not bind IgG2a. Also, FcG2b/1R has been identified as an alloantigen related to the known Ml system described by Mark, et al., J. Immunol. 135: 2635-241 (1985), and Hibbs, et al., Immunogen. 22: 335-348 (1985), a locus on chromosome 1 which specifies products on antigen presenting cells and governs a non H-2T cell proliferative response resulting in intense stimulation

of the mixed lymphocyte reaction. Four alleles of the locus have been identified (Festenstein Transplantation Proceedings 8: 339-342 (1976), and the Ly-17 antigen, which is known to be identical to FcG2b/1R (Holmes, et al., Proc. Natl. Acad. Sci. 82: 7706-7710 (1985) is either closely linked to or determined by this locus.

Given the differences in isotype specificity between macrophage and lymphocyte receptors, as well as the functional heterogeneity thereof, isolation and characterization of cDNAs of macrophage and T cell clones was undertaken. The result of these experiments was isolation of two genes, one of which exists in two different allelic forms, which express proteins with highly homologous extracellular domains which bind IgG, but which possess different transmembrane and cytoplasmic domains. These genes will be referred to as FcαR, FcβR, and $Fc\beta_2R$ hereafter.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the restriction map and sequencing strategy (a) and nucleotide sequence (b) for the gene expressing macrophage FcGα UT = untranslated sequences, S = signal sequence, extracellular domain = E-C, transmembrane domain = TM, and cytoplasmic domain = C.

Figure 2 shows homology of FcG protein to MHC class II protein E . A single dot indicates conservative mutation, while two dots indicate identity.

Figure 3 shows the distribution of FcGα mRNA in various cell lines.

Figure 4 parallels Figure 1 in that it shows the restriction map and sequencing strategy (a), and nucleotide sequence of FcG β_1 cDNA (b).

Figure 5 depicts amino acid alignment of FcGα and FcG β_1 proteins. Overall, there is 95% homology in the extracellular portion.

Figure 6 shows Southern Blot Analysis of DNAs of inbrid mice, and identifies a polymorphism linked to the FcG gene.

Figure 7 shows the distribution of β, transcripts in different cell lines, and provides evidence for a macrophage β_2 transcript.

Figure 8 shows the restriction map and sequencing strategy (a), and the nucleotide sequence (b) of FcG β_2 cDNA.

Figure 9 shows the expression of $FcG\beta_2$ in transfected mouse melanoma cells, via reconstitution of immunoglobulin binding activity.

Figure 10 is a summary of the structure of the IgG2b/G1 FcR cDNA genes obtained from macrophage and T cell lines.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Purification and Amino-Terminal Sequence of $Fc\gamma R$

$Fc\gamma$ receptor was purified from the S49.1 cell line by a slight modification of the published procedure of Mellman, et al. J. Exp. Med. 152: 1048 (1980). Cells (1×10^{10}) grown in suspension culture were lysed in 30 ml of 2% Nonidet P-40 in PBS containing 0.2 TIU aprotinin per ml and 5 mM diisopropylfluorophosphate. The 40,000 xG supernatant from the lysate was applied to a 5 ml column of Sepharose 4B coupled with 2 mg of 2.4G2 IgG per ml of resin. The column was washed first with 10 column volumes of 1% NP-40/0.2% sodium dodecyl sulfate in PBS, and then with 10 column volumes of 10 mM octyl-β-D-thioglucoside in PBS. Protein was eluted with PBS containing 50 mM triethylamine, 10 mM octyl-β-D-thioglucoside, pH 11.0 and rapidly adjusted to neutrality with Tris HCl. The protein was acidified with trifluoroacetic acid, applied to a Supelco C8 2 cm column

and eluted with a gradient of acetonitrile 0.1% trifluoroacetic acid, as described by Pan, et al., J. Chromatog. 297: 13-19 (1984).

The amino terminal 22 amino acids for the S49.1 IgG2b/κ1 receptor were determined on 200 pMoles of the above material using a Waters HPLC system and a microsequencing apparatus (Applied Biosysems model 470A) and found to be THDLPKAVVKLEPPWIQVLKED. The seven amino acids EPPWIQV were chosen for the synthesis of a corresponding oligodeoxynucleotide based on their relatively low degeneracy. After considering preferred codon usage described by Chen, et al., DNA 4: 365-374 (1982) and the ability of G-T base pairing to form, a 20 nucleotide sequence was synthesized that corresponds to the complementary strand of the sequence encoded above. That sequence is 5' ACTTG$^{G}_{T}$ATCCA$^{G}_{T}$GG$^{G}_{T}$GGTTC 3'.

Necleotide and Deduced Portein Sequence of the Macrophage FcγRα

The low degeneracy mixed probe described supra was end-labelled to high specific activity with ^{32}p-γATP and used to screen a mouse macrophage cDNA library constructed to size-fractionated J774 mRNA in the plasmid vector pUC9

(Portnoy, et al., J. Biochem. (1986). A library of 50,000 clones was screened from which 2 positives were identified.

The screening involved a hybridization reaction which included 6X NETS, IX Denhardts, 10^6cpm/ml ^{32}P-end labelled oligonucleotide (10^9cpm/μg) at 45°C for 16 hours. The filters were then washed in 6X SSC at 25° for 5 minutes, followed by a 40°C wash in the buffer described supra for 1 minute, followed by a 1 minute wash in the same buffer at 45°C. Filters were dried, autoradiographed for 6 hours at -70°C with intensifying screens and developed. Positive clones were identified and the filters rewashed at 50°C in 6X SSC for 1 minute and then at 65°C for 1 minute. Positives melt off differentially at the higher temperature and were subjected to colony purification, plasmid preparation and restriction analysis. The clone with the largest insert (a 1300 base pair PstI fragment) was chosen for subsequent analysis. The sequencing strategy for this clone is presented in Figure 1a with the nucleotide sequence and deduced amino acid sequence in Figure 1b. An open reading frame of 782 nucleotides was found, beginning with an ATG at position 64 and terminating at position 846. The predicted signal peptidase cleavage site is indicated by an arrow and is assigned based on the consensus rules for such

sequences (Von Heijne, Eur. J. Biochem 133: 17-21 (1983). 19 amino acids, encoded from nucleotides 160-210, are identical to amino acids 3-22 of the S49.1 sequence presented above, with the exception of position 12 which is a glutamic acid residue in S49.1 and an aspartic acid in J774. The discrepancy in the first three amino acids and position 12 resulted from the heterogeneity between the macrophage and T cell proteins, described infra. A 30 amino acid signal sequence is predicted, and is numbered -30 to -1 with a hydrophobic core overlined. Predicted signal peptidase cleavage site is indicated by the arrow between -1 and 1 following Von Heijne, supra. N-linked glycosylation sites are boxed and cysteine residues circled. The deduced protein sequence contains two regions of hydrophobic amino acid residues, overlined in Figure 1b. These regions encode the putative signal sequence (nucleotides 64-153) and a transmembrane anchor sequence (nucleotides 709-769). An extracelluar domain of 185 amino acids is expected for the mature protein which contains 4 potential N-linked glycoslyation sites (boxed in Figure 1b) as well as four cysteine residues which could form 2 intra-chain disulfide bonds. A serine and threonine rich region is encoded from amino acids 155-185, just preceding the transmembrane

domain, in which 30% of the residues are represented by these two amino acids. A cytoplasmic domain of 26 amino acids is predicted from this sequence. The primary sequence predicts a molecular weight of 30,040 daltons, which would then be subject to glycoslyation at the four N-linked sites described, and possibly O-linked sites as well.

The extracellular domain described above consists of two internally repeated sequences. Amino acids 25-75 of the predicted mature protein show homology to amino acids 100-155. These homologies cluster around the cysteine residues, suggesting a structural repeating domain. These data suggest that the extracelluar domain consits of a repeated domain defined by the cysteine residues. Comparison of this sequence to the protein sequence databanks revealed significant homology to immunoglobulin molecules, MHC class I and class II proteins, β2 microglobulin and other members of this supergene family. Homology of the extracelluar domain with a rabbit V region has been deomonstrated showing clusters of homology centered about the cysteine residues in both proteins. This homolog suggests that this Fc receptor contains two immunoglobulin-like domains, each consisting of a potential disulfide loop of 42 amino acids within a domain of 70-80 amino acids.

The most significant homology we found for this FcR is to the MHC class II protein Eβ, with 32% identity over a 91 amino acid region, as shown in Figure 2a. Random shuffling of these two sequences, using the program rdf (Lipman, et al., Science 227: 1435-1441 (1985), indicated that the optimized alignment shown in Figure 2a is highly significant, at 5 standard deviations above the mean. This homology to E β occurs in the β_2 domain (Figure 2b) which itself is an immunoglobulin-like domain. Homology is also apparent by this analysis in the transmembrane domain of these two proteins.

mRNA extracted from a variety of cell lines was analyzed for the presence of message corresponding to the cDNA cloned from the J774 cell line, referred to as the α gene. The procedure used generally followed Chitgwin, et al., Biochem. 18: 5294 (1979), and Lehrach, et al., Biochem. 16: 4743-4751 (1977). Briefly, 1 ug of poly A^+ RNA was fractionated on agarose-formaldehyde gels transferred to nitrocellulose and hybridized under stringent conditions either with the complete α probe (a) or a probe constructed to the 5'α sequences (b). As can be seen in Figure 3, a broad band of hybridization is detected in the macrophage lines P 388D1, WEHI, 3A, RAW 264.7 and J774. A T cell line

S49.1, which reacts with the FcγR monoclonal antibody 2.4G2 contains a higher molecular weight mRNA species. P388 demonstrates two RNA species of equivalent abundance, while WEHI 3A has a major band migrating faster than 18S and a band of much lower abundance migrating slower than 18S. 2.4G2 negative lines CL.7 (fibroblast), L-cell (fibroblast) and L51789 (T cell) do not contain an α transcript. The cell type specificity of expression of the α gene was apparent when a probe was constructed to the 5' sequences of the cDNA and used to examiner the same macrophage and T-cell RNAs (Figure 3b). No transcript is detected in the S49.1 cell line with this probe and only a single species is seen in P388. Similarly, the lower abundance species migrating slower than 18S in WEHI 3A is not detected with this probe. Similar results were obtained when a 3' probe was constructed from the α cDNA (Sca-Pst, data not shown). These results show that the T cell line S49.1 and the macrophage-like lines P388 and WEHI 3A contained cross-hybridizing RNA species when probed with the complete probe which are not homologous to the macrophage α transcript on their 5' and 3' ends. Analysis of two other T cell lines EL-4 and K-36 demonstrated transcripts of similar size to that found in S49.1 (data not shown). To identify

these transcripts and the genetic basis for their expression, a cDNA library was constructed to size-fractionated S49.1 mRNA and probed with the complete cDNA α probe. Positive clones were identified at a frequency of .1% and were characterized by restriction mapping and DNA sequence analysis.

Figure 4a presents the physical map and sequencing strategy for the T cell transcript, referred to as β_1, and 4b presents the nucleotide sequence and predicted amino acid sequence for this protein. The procedures used were the same as those described supra for obtaining the physical map and sequence of macrophage DNA. A single open reading frame was found, beginning with an ATG at nucleotide 340 and terminating at nucleotide 1326. The predicted molecular weight of this protein before modification is 36,750 daltons. Beginning at nucleotide 427 (position +1) 22 amino acids are encoded which are identical to the determined amino terminal sequence for this protein. The sequence which precedes this N terminus encodes a signal sequence with a characteristic hydrophotic core (overlined), which bears no homology to the signal sequence of the α transcript (see Figure 1b). The extracellular domain has 95% identity with the α sequence, as shown in Figure 5, beginning at

amino acid 4 of the β_1 sequence and continuing through amino acid 174. A transmembrane and cytoplasmic domain are predicted for the β_1 sequence, which bear no homology to the analogous domains of the sequence. No sequence homology is found in the 5' and 3' untranslated domains of these two transcripts.

Four additional tryptic peptides were sequenced for the S49.1 FcγR. A 10 amino acid sequence SQVQASYTFK was confirmed at positions 50-59 of the β_1 sequence; the 8 amino acid sequence ISFFHNEK was confirmed at positions 120-127; the 13 amino acid sequence EMGETLPEEVGEY was present at positions 222-235, and finally the 13 amino acid sequence TEAENTITYSLLK was confirmed at positions 271-283.

Since both α and β_1 sequences were obtained from cell lines derived from Balb/c mice, the 5% sequence variation in the highly conserved extracelluar domain could not arise from allelic variation. To confirm that the β_1 transcript was derived from a second gene, Southern blot analysis of DNA obtained from different inbred strains of mice was used to map the $^{\alpha}$ and $^{\beta}$ genomic sequences. As seen in Figure 6, DNA cut with Taq 1 and probed with the complete α probe detects a polymorphism associated with this gene, giving rise to three different restriction fragment lengths. This

polymorphism resides 3' of the α gene, as determined by reprobing these blots with 5' and 3' probes (data not shown). However, when these same DNA samples are probed with the complete β_1 cDNA probe (Figure 6b), the 3' polymorphic fragment associated with the α gene is replaced by 2.4 kb non-polymorphic fragment. These data suggest that the α and β; transcripts are derived from different genes, which contain a highly conserved sequence encoding an extracellular domain. Hybridization with both the α and β probes under conditions of low stringency (25% formamide, 10% dextran sulphate, 5X SSC at 40°C; final wash = .2X SSC, .1% SDS 40°C) revealed an additional cross-hybridizing restriction fragment, perhaps suggestive of a third member of this gene family (not shown).

Expression of the β Gene in T cell and Macrophage Lines

Figure 7a presents the result of RNA blot analysis of macrophage and T cell lines probed with the β_1 cDNA probe. This probe is expected to detect the S49.1 transcript, as well as cross-hybridize to the α transcript in macrophage lines. However, comparing the results of this blot with that in Figure 4a it is clear that a different pattern of transcripts are detected with different abundance. In

particular WEHI 3A and P388 demonstrates two transcripts of comparable abundance one of which has the same apparent mobility as the S49.1 transcript. This is confirmed by using a 5' β_1 probe, shown in Figure 7b, constructed to the 5' untranslated region of this transcript in which no homology to the α gene is found. Transcripts are detected in macrophage lines with this probe, demonstrating that the β gene is expressed in macrophages, resulting in a different transcript size in J774, RAW and P388D1 than what is found in the S49.1 T cell line. To investigate these macrophage β transcripts, the J774 cDNA library was screened with both unique and common sequences derived from the β gene. Restriction enzyme mapping of the clones obtained demonstrated that these clones differed from the β_1 transcript of the S49.1 cell line in lacking an Xmn site. The physical map and sequencing strategy for the macrophage β transcript, referred to as β_2 is shown in Figure 8a and the nucleotide sequence and deduced amino acid sequence in Figure 8b. The procedure followed for obtaining these was the same as that given supra.

Comparison of the β_1 and β_2 Transcripts: Evidience for a T Cell Specific Splice in the Cytoplasmic Domain

The β_1 and β_2 sequences are identical throughout their length, both for coding and noncoding sequencies, with the exception of a 138 nucleotide insertion found in the β_1 sequence (nucleotides 1066-1204, indicated by the upward arrows in Figure 4b) which occurs after nucleotide 783 in the β_2 sequence. This sequence results in a 46 amino acid insertion in the cytoplasmic domain of the β_1 transcript. This insertion accounts for the larger transcript found in S49.1 as well as in the T cell lines EL-4 and K-36 (data not shown). RNase protection using a T7 RNA polymerase generated radiolabelled RNA probe specific for the β_1 gene (Xmn-Pst, see Figure 4a) detected a 500 nucleotide protected fragment, specific for the β_1 gene, in the S49.1 and K-36 T cell lines and not in the macrophage line P388D1. In addition to the 500 bp protected fragment, a 362 bp fragment was detected in S49.1 and K-36 which comigrated with the P388D1 macrophage protected fragment, consistent with the size expected to be protected by the β_2 transcript. These results suggest that the β gene is transcribed in T cells and macrophages. In T cells, however, a transcript with a 138 bp insertion is found, which is most simply explained by

an alternative splicing pathway which gives rise to an additional exon in the T cell FcγRβ. The predicted molecular weight of the β_2 protein is 31,886 prior to any post-translational modification.

Functional Domains of the FcγR: Expression in Transfected Cell Lines

In order to begin to assess the functional role of the structural heterogeneity described for this Fc receptor and to ascertain if more than one polypeptide chain was necessary for ligand binding, Fc receptor negative cell lines were transfected with these cDNA clones. Expression was achieved by cloning the coding sequence of the β_1 or β_2 cDNAs into an expression vector (pcEXV-3) as described by Miller, et al., J. Immunol. 134: 4212-4217 (1985) which utilizes the SV40 early promoter to achieve transcription of the cloned sequences. B78H1 mouse melanoma cells were cotransfected with the plasmid constructions and pGCcos3neo, which confers resistance to the drug G418 (Southern, et al., J. Mol. Appl. Genet. 1: 327-341 (1982). After 10 days in G418 containing medium colonies were screened by rosetting with human erythrocytes conjugated with the monoclonal

antibody 2.4G2, as described by Albino, et al., Mol. & Cell Biol. 5: 692-697 (1985). Positive cells were cloned and then tested for Fcγ receptor activity.

Figure 9 presents the results obtained with the β_2 cDNA cloned into the expression vector. Stable lines expressing the 2.4G2 epitope avidly bound sheep red blood cells opsonized with rabbit anti-SRBC, which is diagnostic of Fcγ receptor function. In addition, this binding is blocked in a concentration dependent fashion by the monoclonal antibody 2.4G2, demonstrating the specificity of binding to this receptor. Transfectants which were obtained with the β_1 insert in the expression vector demonstrated the same pattern of binding. Controls with untransfected B78H1 cells, transfected cells in which the FcγR β sequences were in the reverse orientation with respect to the SV40 promoter as well as studies with SRBCs not coated with antibody all gave negative results (data not shown). These experiments indicate that the protein expressed by these cDNA clones is able to be displayed on the cell surface and mediate the binding of antibody-antigen complexes in a specific manner. Experiments with other cell lines, such as mouse L cells or monkey cos cells suggests that the ability of these sequences to specify an Fc receptor is not cell type specific.

Two genes have been identified that encode Fc γ receptors. One of these, referred to as α, is expressed in macrophage cell lines and peritoneal macrophages. The second gene, referred to as β, is expressed in both macrophage and in T-cell lines. These genes enclode transmembrane proteins with two repeated N-terminal domains each containing two glycosylation sites, and a potential intrachain disulfide loop of 42-45 amino acids. This predicted structure is consistent with in vivo labeling studies, which have suggested the presence of 4 N-linked glycosylation sites. While the extracellular domains of the γ and β genes are 95% homologous, the transmembrane and cytoplasmic domains encoded by the two genes are totally different, which suggests that the different functions of lymphocyte and macrophage Fc γ receptors derives in part from different signaling mechanisms.

There is, however, still another level of complexity in this gene system, since there appear to be cell-specific splicing mechanisms that may result in altered protein products. Although the β gene is transcribed in both T cell lines and in macrophages, analysis of the T cell specific transcript (β_1) revealed an additional 138 nucleotides, which result in a 46 amino acid insertion in

the cytoplasmic domain of the T cell lines Fcγ receptor. RNase protection experiments have shown that the β_1 transcript is seen in T cell lines, and not in the J774, P388D1 or RAW macrophage cell lines and most probably arises from an alternative splicing pathways for the β gene. The early macrophage lines P388 and WEHI 3A have β transcripts of the same apparent mobility as the T cell lines which may suggest that β_1 splicing is developmentally regulated during macrophage maturation. The consequences of the insertion are not understood, but it is possible that the longer cytoplasmic domain in the β_1 FcγR interacts differently with cytoplasmic or membrane proteins involved in signal transduction. The structures of the three transcripts derived from the α and β genes are summarized in Fig. 10.

The sequences obtained for these receptors demonstrate that the Fcγ receptor belongs to the immunoglobulin supergene family, as does the poly IgA receptor, which functions in the transport of IgA across epithelial cells (Mostov, et al., Nature 308: 37-43 (1984). Apart from the overall immunoglobulin homology, several other significant homologies were identified for the FcγR. The extracellular domain of the α and β genes are most homologous to the MHC class II protein Eβ in its β_2 domain. The poly Ig

receptor, by contrast, has little significant homology to the FcγR receptor described here, suggesting that it is more distantly related to the FcγR receptor than are class II determinants. The other significant homology detected for the transmembrane domain of the α chain, but not the β chain, is with one of the transmembrane domains of the chain of the acetylcholine receptor. Young et al., Proc. Natl. Acad. Sci. 80: 1636-1640 (1983) and Nelson, et al., J. Clin. Invest 76: 500-507 (1985) have reported ion channel activity in response to binding of ligand to FcγR receptors. The precise role of the various structural domains of these Fc receptor proteins is under investigation.

Applications of the foregoing aspects of the invention will be recognized by one skilled in the art. For example, in some individuals with immune disorders, the cause can be traced to cells and organs which do not express the necessary Fc receptor proteins, and therefore do not mount a sufficient or complete response to infections. Artificial organs are contemplated, which either incorporate therein Fc receptor proteins produced in vitro by appropriate transformed cells in culture, or organs which are "seeded", with physiologically acceptable transformed cells which also produce the necessary protein.

A second application will be seen in the use of DNA of this invention to obtain human DNA corresponding to the DNA described herein. One may now follow established techniques of DNA hybridization, e.g., to locate and hybridize human Fc receptor protein expressing DNA with murine "probes". The details of such hybridization technologies are known to those skilled in the art and need not be elaborated upon further.

While there have been described what are at present considered to be the preferred embodiments of this invention, it will be obvious to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is, therefore, aimed to cover all such changes and modifications as fall within the true spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. Substantially pure nucleotide sequence expressing Fc receptor protein.

2. Nucleotide sequence of claim 1, wherein said receptor protein is $Fc\gamma$ receptor protein.

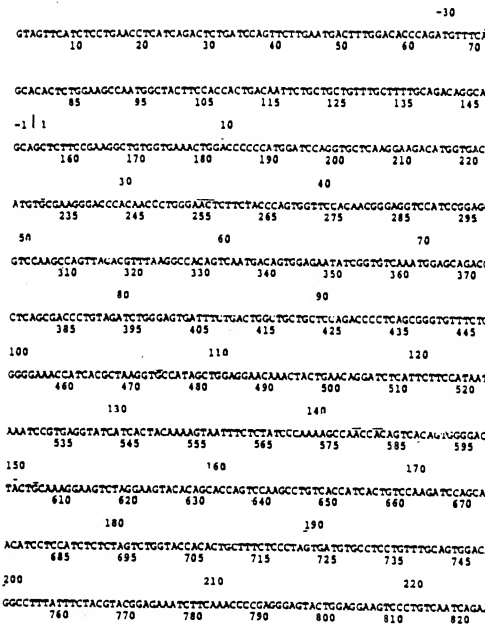
3. Nucleotide sequence of claim 2, wherein said receptor protein is $Fc\gamma^{a}$ receptor protein.

4. Nucleotide sequence of claim 2, wherein said receptor protein is $Fc\gamma\beta$ receptor protein.

5. Nucleotide sequence of claim 4, wherein said $Fc\gamma\beta$ receptor protein is $Fc\gamma\beta$ receptor protein.

6. Nucleotide sequence of claim 4, wherein said Fc receptor protein is $Fc\gamma\beta_2$ receptor protein.

7. Nucleotide sequence of claim 3, comprising sequence:



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                    230
CACCAGGCTCCTCAAGACAAGTGACACCCCATCCATCCTATGGCAAAACATACGATGTTTTGGTGGCAGCAGCAA
      835       845       855       865       875       885       895

CTTTTCAGCCACACAGCCTTCCTTTGAAAGCAACTTACAAGCAGGCCGGGATGTTTGGTTCTTCAATCACAACGA
      910       920       930       940       950       960       970

CTTAGGATCACCAGTTCAAGGCTTGCTGGGTCACACAGAGAGAGTGAGTGCAAGTCTAGCCTGGATAACCCAGTG
      985       995      1005      1015      1025      1035      1045

AGATCCTGGGTTTAGGCGGCTCATCAGGAAAGAGAACCTGTTGCTAATCTCACAAACAAGATGCCTACTGCCCAT
     1060      1070      1080      1090      1100      1110      1120

GTGGCCAAAGGAGAGAACAAGGTCCTGGAAGTTGTCCTCTGACCTCCACCATCCACCATGGCAGGTGCACACAAT
     1135      1145      1155      1165      1175      1185      1195

AAATTAAAATGTCATGTATATTTTTAAACAAGAGACAGGGGCAGGCTAAGGGTTGATGGCATAGCTGTTATCCAG
     1210      1220      1230      1240      1250      1260      1270

TACACATAATGCCCTGGGTTTGACCTCCTATAATAAAGCAAAAAAAAAAAAAAAAAAAAAAAA
     1285      1295      1305      1315      1325      1335
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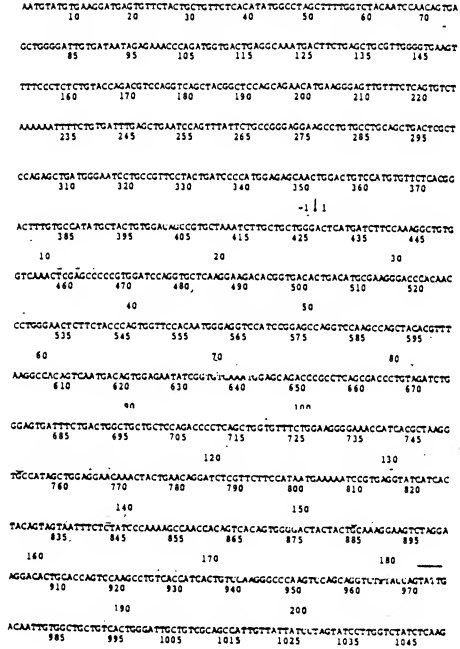
8. Nucleotide sequence of claim 5, comprising sequence:



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  210                         220                         230
AAAAAGCAGGTTCCAGCTCTCCCAGGAAACCCTGATCAGAGGGAAATGGGAGAAACCCTTCCAGAGGAAGTAGGT
      1060        1070        1080        1090        1100        1110        1120

                  240                         250
GAGTACAGACAACCCTC                TGCCTGTCAGCCCAGGGCTCCATCTGGACTGGAGCCAACAAGCAGCAGC
      1135        1145        1155        1165        1175        1185        1195

  260                         270                         280
CCATACAATCCTCCTGATCTGGAAGAAGCTGCCAAAACTGAGGCTGAGAATACGATCACCTACTCACTTCTCAAG
      1210        1220        1230        1240        1250        1260        1270

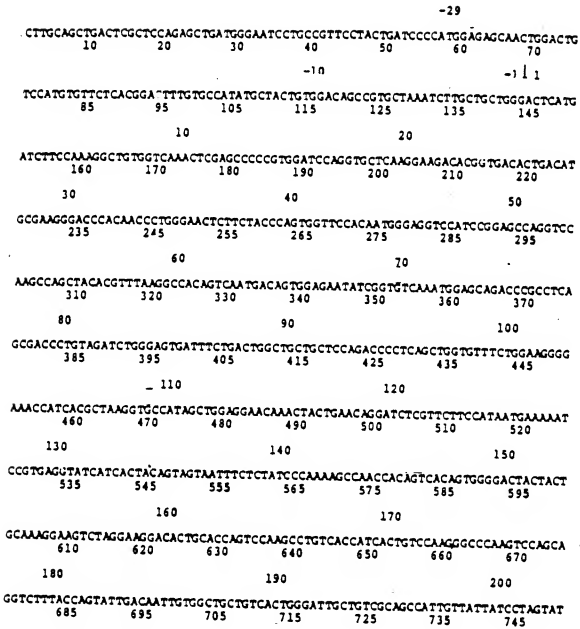
                  290                         300
CATCCCGAAGCCCTGGATGAAGAAACAGAGCATGATTACCAGAACCACATTTAGTCTCCCTTGGCATTGGGAAAA
      1285        1295        1305        1315        1325        1335        1345

GCAAGCCAGAAAGGCCAGGATCTAGTGTCTCCTGGTCCAAGGGATGCTGTAGATATTAAAGAAAACATCCAGAGT
      1360        1370        1380        1390        1400        1410        1420

CACTTCTGTGAGTCCTGAAACCAACAGACACTACGAGATTGGTTCCCAATGGTTGACTGTACTAATGACTCCCAT
      1435        1445        1455        1465        1475        1485        1495

AACTTACAGCTTCCCAACTCAAGACTCTTCTGCTATCGATCCACACTGCCACTAAAATTAATCAACTTACTGCC
      1510        1520        1530        1540        1550        1560        1570
```

9. Nucleotide sequence of claim 6, comprising sequence:



```
                    210                                 220
CCTTGGTCTATCTCAAGAAAAAGCAGGTTCCAGACAATCCTCCTGATCTGGAAGAAGCTGCCAAAACTGAGGCTG
      760       770       780       790       800       810       820

   230                              240                              250
AGAATACGATCACCTACTCACTTCTCAAGCATCCCGAAGCCCTGGATGAAGAAACAGAGCATGATTACCAGAACC
      835       845       855       865       875       885       895

  254
   I
ACATTTAGTCTCCCTTGGCATTGGGAAAAGCAAGCCAGAAAGGCCAGGATCTAGTGTCTCCTGGTCCAAGGGATG
      910       920       930       940       950       960       970

CTGTAGATATTAAAGAAAACATCCAGAGTCACTTCTGTGAGTCCTGAAACCAACAGACACTACGAGATTGGTTCC
      985       995      1005      1015      1025      1035      1045

CAATGGTTGACTGTACTAATGACTCCCATAACTTACAGCTTCCCAACTCAAGACTCTTCTGCTATCGATCCACAC
     1060      1070      1080      1090      1100      1110      1120

TGCCACTAAAATTAATCAACTTACTGCCGTTAAGAGA
     1135      1145      1155
```

10. Substantially pure Fcγ receptor protein.

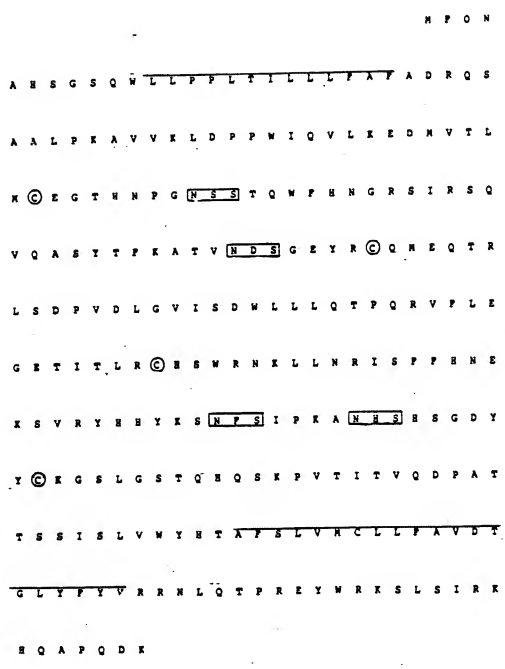
11. Substantially pure Fcγ receptor protein of claim 10, wherein said protein is Fcγ^{α} protein.

12. Substantially pure Fcγ receptor proteins of claim 10, wherein said protein is Fcγ^{β} protein.

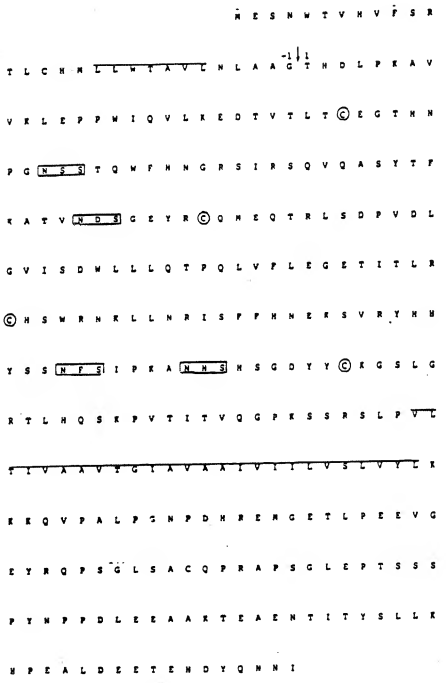
13. Substantially pure Fcγ^{β} receptor protein of claim 12, wherein said protein is Fcγ^{β}_{1} protein.

14. Substantially pure Fcγ^{β} receptor protein of claim 12, wherein said protein is Fcγ^{β}_{2}.

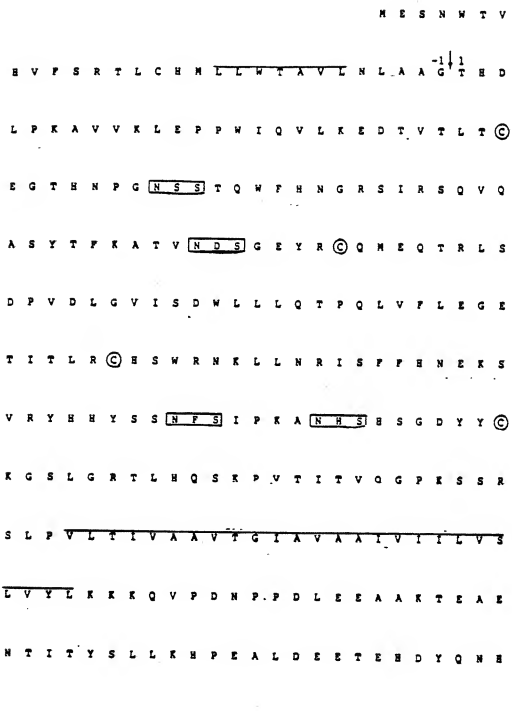
15. Substantially pure protein of claim 11, comprising amino acid sequence:



16. Substantially pure protein of claim 13, comprising amino acid sequence



17. Substantially pure protein of claim 14, comprising amino acid sequence:



18. A method of obtaining substantially pure Fc receptor protein comprising transforming a eukaryotic cell with substantially pure nucleotide sequence expressing an Fc receptor protein selected from the group consisting of $Fc\alpha$, $Fc\beta_1$ and $Fc\beta_2$ receptor protein culturing said transformed cell under conditions favoring expression purifying of said protein, and said expressed protein.

19. Vector which expresses Fc receptor protein, comprising a substantially pure nucleotide seqiemce expressing Fc receptor protein selected from the group consisting of Fc , $Fc\beta$, and $Fc\beta_2$ ligated into a portion of carrier DNA.

20. Vector of claim 19, wherein said vector is pcEXV-3.

21. Eukaryotic cell line transformed with a foreign nucleotide sequence which expresses an Fc receptor protein selected from the group consisting of $Fc\alpha$, $Fc\beta$, and $Fc\beta_2$.

22. An immunoglobulin receptor organ analog which comprises a physiologically acceptable cell line of claim 21.

23. A method for obtaining substantially pure human DNA which expresses human Fc receptor proteins comprising contacting a sample containing said substantially pure human DNA with a sample of mouse DNA which expresses mouse Fc receptor protein under conditions favoring hybridization between said mouse DNA and complementary human DNA, treating said sample to separate hybridized DNA from unhybridized DNA and removing said mouse DNA from said hybrid.

24. Method of claim 23, wherein said DNA expresses a receptor protein selected from the group consisting of $Fc\gamma$, $Fc\gamma\alpha$, $Fc\gamma\beta_1$, and $Fc\gamma\beta_2$.

25. Substantially pure human DNA obtained by the method of claim 23.

FIGURE 1a

$\gamma_{2b}/\gamma_1 F_c R\ \alpha$ (J774)

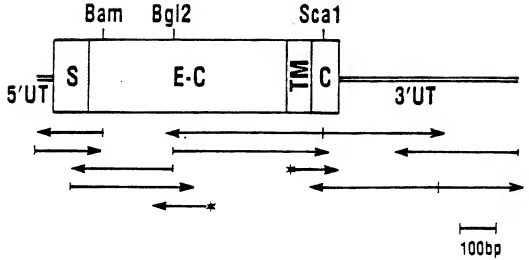


Figure 1b

SUBSTITUTE SHEET

Figure 1b, cont.

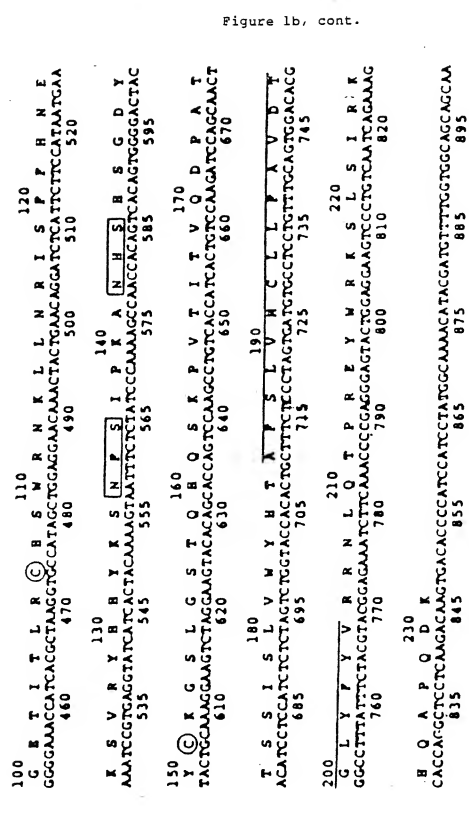


Figure 1b, cont.

```
CTTTTCAGCCACACAGCCTTCCTTTGAAAGCAACTTACAAGCAGGCCGGGATGTTTGGTCTCTCAATCACAACGA
      910       920       930       940       950       960       970

CTTAGGATCACCAGTTCAAGGCTTGCTGGGTCACACAGAGAGAGTGAGTGCAAGTCTAGCCTGGATAACCCAGTG
      985       995      1005      1015      1025      1035      1045

AGATCCTGGGTTTAGGCCGCTCATCAGGAAAGAGAACCTGTGCTAATCTCACAAACAAGATGCCTACTGCCCAT
     1060      1070      1080      1090      1100      1110      1120

GTGGCCAAAGGAGAGAACAAGGTCCTGGAAGTTGTGCCTCTGACCTCCACCAATCCACCATGGCAGGTGCACAAT
     1135      1145      1155      1165      1175      1185      1195

AAATTAAAATGTCATGTATATTTTTAAACAAGACAGGGGCAGGCTAAGGGTTGATGGCATAGCTGTTATCCAG
     1210      1220      1230      1240      1250      1260      1270

TACACATAATGCCCTGGGTTTGACCTCCTATATAAAGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
     1285      1295      1305      1315      1325      1335
```

Figure 2a

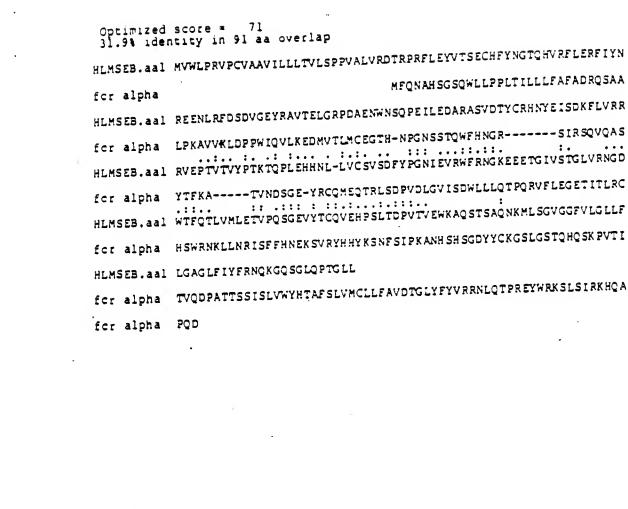
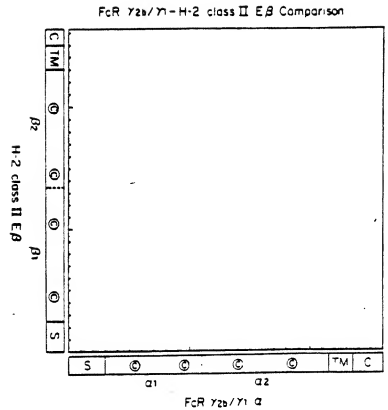
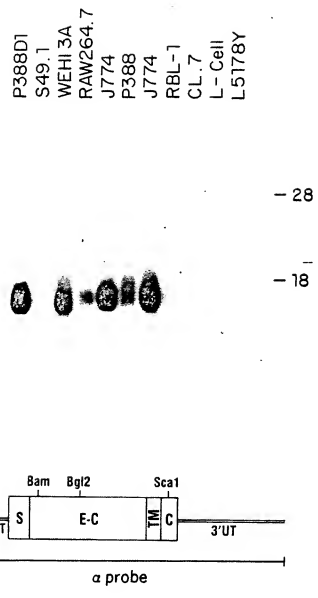

Optimized score = 71
31.9% identity in 91 aa overlap
HLMSEB.aal MVWLPRVPCVAAVILLLTVLSPPVALVRDTRPRFLEYVTSECHFYNGTQHVRFLERFIYN
fcr alpha MFQNAHSGSQWLLPPLTILLLFAFADRQSAA
HLMSEB.aal REENLRFDSDVGEYRAVTELGRPDAENWNSQPEILEDARASVDTYCRHNYEISDKFLVRR
fcr alpha LPKAVVKLDPPWIQVLKEDMVTLMCEGTH-NPGNSSTQWFHNGR-------SIRSQVQAS
HLMSEB.aal RVEPTVTVYPTKTQPLEHHNL-LVCSVSDFYPGNIEVRWFRNGKEEETGIVSTGLVRNGD
fcr alpha YTFKA-----TVNDSGE-YRCQMSQTRLSDPVDLGVISDWLLLQTPQRVFLEGETITLRC
HLMSEB.aal WTFQTLVMLETVPQSGEVYTCQVEHPSLTDPVTVEWKAQSTSAQNKMLSGVGGFVLGLLF
fcr alpha HSWRNKLLNRISFFHNEKSVRYHHYKSNFSIPKANHSHSGDYYCKGSLGSTQHQSKPVTI
HLMSEB.aal LGAGLFIYFRNQKGQSGLQPTGLL
fcr alpha TVQDPATTSSISLVWYHTAFSLVMCLLFAVDTGLYFYVRRNLQTPREYWRKSLSIRKHQA
fcr alpha PQD

Figure 2b


FcR γ2b/γ1 - H-2 class II Eβ Comparison
H-2 class II Eβ
β2
β1
C
TM
S
S
TM
C
α1
α2
FcR γ2b/γ1 α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WEHI 3A
RAW264.7
J774
P388
J774
RBL-1
CL.7
L-Cell
L5178Y
28
18
Bam
Bgl2
Sca1
5'UT
S
E-C
TM
C
3'UT
α probe

Figure 3A

P388D1
S49.1
WEHI 3A
RAW264.7
J774
RBL-1
P388

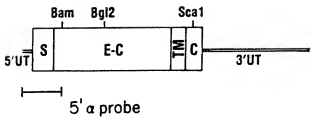


Figure 3B

Figure 4a

$\gamma_{2b}/\gamma_1 F_c R\ \beta_1$ (S49.1)

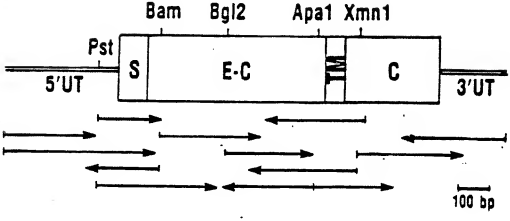


Figure 4b

```
GCTGGGGATTGTGATAATAGAGAAACCCAGATGGTGACTGAGGCAAATGACTTCTGAGCTGCGTTGGGGTCAAGT
     85        95        105       115       125       135       145

TTTCCCTCTCTGTACCAGACGTCCAGGTCAGCTACGGCTCCAGCAGAACATGAAGGGAGTTGTTTCTCAGTGTCT
     160       170       180       190       200       210       220

AAAAAATTTTCTGTGATTTGAGCTGAATCCAGTTTATTCTGCCGGGAGGAAGCCTGTGCCTGCAGCTGACTCGCT
     235       245       255       265       275       285       295

                                       -29                         -20
                                        M  E  S  N  W  T  V  H  V  F  S  R
CCAGAGCTGATGGGAATCCTGCCGTTCCTACTGATCCCCATGGAGAGCAACTGGACTGTCCATGTGTTCTCACGG
     310       320       330       340       350       360       370

                  -10                            -1|1
T  L  C  H  M  L  L  W  T  A  V  L  N  L  A  A  G  T  H  D  L  P  K  A  V
ACTTTGTGCCATATGCTACTGTGGACAGCCGTGCTAAATCTTGCTGCTGGGACTCATGATCTTCCAAAGGCTGTG
     385       395       405       415       425       435       445

  10                              20                             30
 V  K  L  E  P  P  W  I  Q  V  L  K  E  D  T  V  T  L  T (C) E  G  T  H  N
GTCAAACTCGAGCCCCCGTGGATCCAGGTGCTCAAGGAAGACACGGTGACACTGACATGCGAAGGGACCCACAAC
     460       470       480       490       500       510       520
```

Figure 4b, cont.

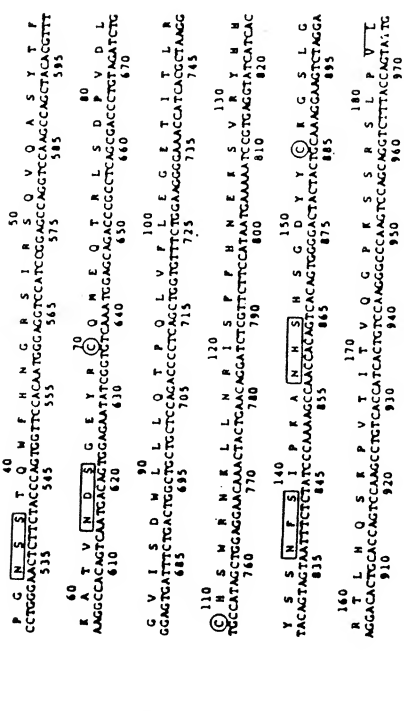


Figure 4b, cont.

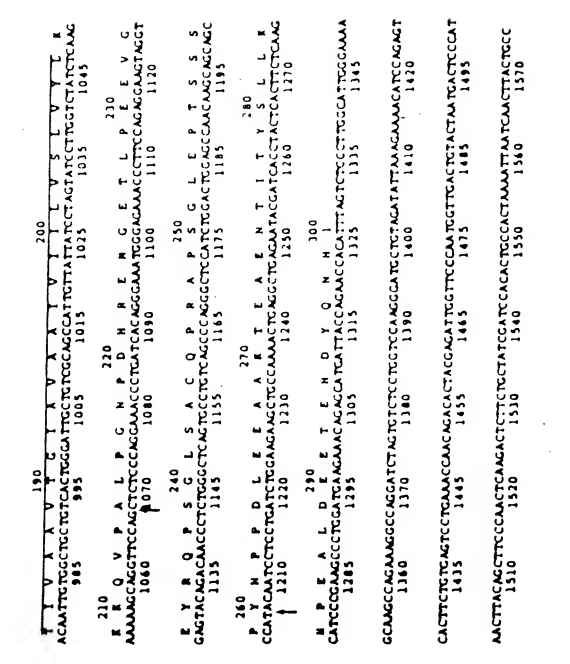
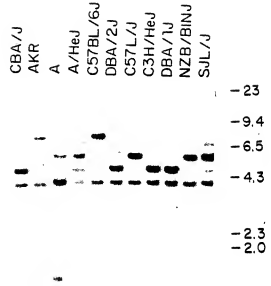


Figure 5

```
fcr beta 1 MESNKTVHVFSRTLCHMLLWTAVLNLAAGTHDLPKAVVKLEPPWIQVLKEDTVTLTCEGT
fcr alpha    MFQNAHSGSQWLLPPLTILLLFAFADRQSAALPKAVVKLDPPWIQVLKEDMVTLMCEGT
                                                                    
fcr beta 1 HNPGNSSTQWFHNGRSIRSQVQASYTFKATVNDSGEYRCQMEQTRLSDPVDLGVISDWLL
fcr alpha  HNPGNSSTQWFHNGRSIRSQVQASYTFKATVNDSGEYRCQMEQTRLSDPVDLGVISDWLL
fcr beta 1 LQTPQLVFLEGETITLRCHSWRNKLLNRISFFHNEKSVRYHHYSSNFSIPKANHSHSGDY
fcr alpha  LQTPQRVFLEGETITLRCHSWRNKLLNRISFFHNEKSVRYHHYKSNFSIPKANHSHSGDY
fcr beta 1 YCKGSLGRTLHQSKPVTITVQGPKSSRSLPVLTIVAAVTGIAVAAIVIILVSLVYLKKKQ
fcr alpha   YCKGSLGSTQHQSKPVTITVQDPATTSSISLVWYHTAFSLVMCLLFAVDTGLYFVVRRNL
fcr beta 1 VPALPGNPDHREMGETLPEEVGEYRQPSGLSACQPRAPSGLEPTSSSPYNPPDLEEAAKT
fcr alpha  QTPREYWRNSLSIRKHQAPQD
fcr beta 1 EAENTITYSLLKHPEALDEETEHDYQNH
```



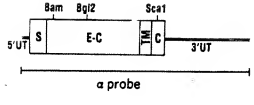
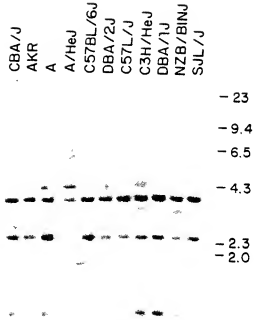


Figure 6A

SUBSTITUTE SHEET


CBA/J
AKR
A
A/HeJ
C57BL/6J
DBA/2J
C57L/J
C3H/HeJ
DBA/1J
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23
9.4
6.5
4.3
2.3
2.0

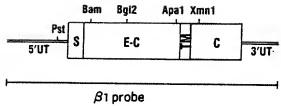

Bam
Bgl2
Apa1
Xmn1
Pst
S
E-C
C
5'UT
3'UT
β1 probe

Figure 6B

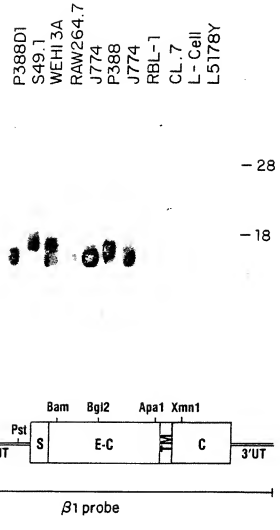


Figure 7A

SUBSTITUTE SHEET

P388D1
S49.1
WEHI 3A
RAW264.7
J774
RBL-1
P388

−28

−18

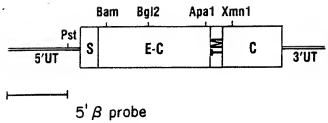


Figure 7B

SUBSTITUTE SHEET

Figure 8a

$\gamma_{2b}/\gamma_1 F_c R\ \beta_2$

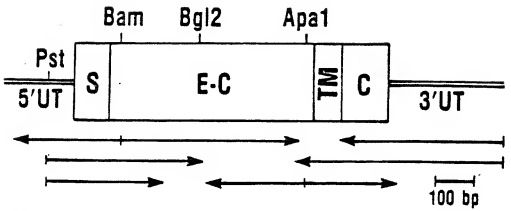
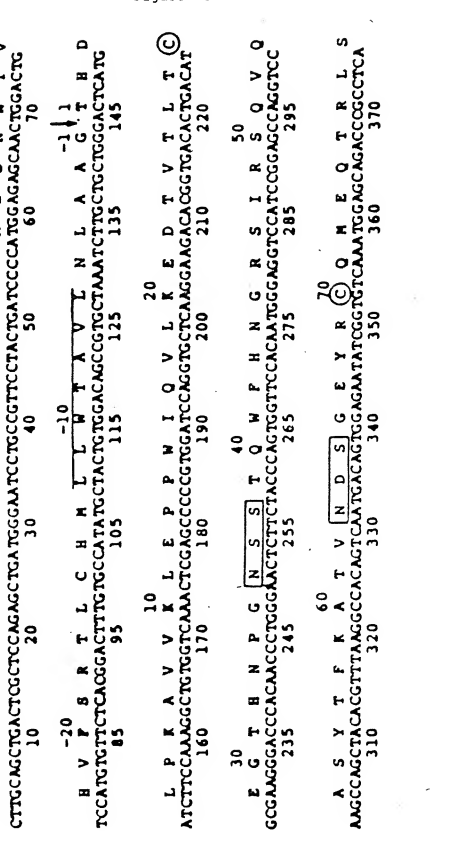


Figure 8b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-20 -10 -1 1
H V P S R T L C H M L L W T A V L N L A A G T H D
TCCATGTGTTCTCAGGACTTTGTGCCATATGCTACTGTGGACAGCCGTGCTAAATCTTGCTGCTGGGACTCATG
85 95 105 115 125 135 145
10 20
L P K A V V K L E P P W I Q V L K E D T V T L T
ATGTTCCAAAGGCTGTGGTCAAACTCGAGCCCCCGTGGATCCAGGTGCTCAAGGAAGACACGGTGACACTGACAT
160 170 180 190 200 210 220
30 40 50
E G T H N P G N S S T Q W F H N G R S I R S Q V Q
GCGAAGGACCCACAACCCTGGGAACTCTTCTACCCAGTGGTTCCACAATGGAGGTCCATCCGGAGCCAGGTCC
235 245 255 265 275 285 295
60 70
A S Y T F K A T V N D S G E Y R Q M E Q T R L S
AAGCCAGCTACACGTTTAAGGCCACAGTCAATGACAGTGGAGAATATCGGTGTCAAATGGAGCAGACCGCCTCA
310 320 330 340 350 360 370

Figure 8b, cont.

SUBSTITUTE SHEET

Figure 8b, cont.

```
                210                                  220
 L  V  Y  L  K  K  K  Q  V  P  D  N  P  P  D  L  E  E  A  A  K  T  E  A  E
CCTTGGTCTATCTCAAGAAAAAGCAGGTTCCAGACAATCCTCCTGATCTGGAAGAAGCTGCCAAAACTGAGGCTG
      760        770       780       790       800       810       820

   230                      240                       250
 N  T  I  T  Y  S  L  L  K  H  P  E  A  L  D  E  E  T  E  H  D  Y  Q  N  H
AGAATACGATCACCTACTCACTTCTCAAGCATCCCGAAGCCCTGGATGAAGAAACAGAGCATGATTACCAGAACC
      835        845       855       865       875       885       895

 254
 I
ACATTTAGTCTCCCTTGGCATTGGGAAAAGCAAGCCAGAAAGGCCAGGATCTAGTGTCTCCTGGTCCAAGGGATG
      910        920       930       940       950       960       970

CTGTAGATATTAAAGAAAACATCCAGAGTCACTTCTGTGAGTCCTGAAACCAACAGACACTACGAGATTGGTTCC
      985        995      1005      1015      1025      1035      1045

CAATGGTTGACTGTACTAATGACTCCCATAACTTACAGCTTCCCAACTCAAGACTCTTCTGCTATCGATCCACAC
     1060       1070      1080      1090      1100      1110      1120

TGCCACTAAAATTAATCAACTTACTGCCGTTAAGAGA
     1135       1145      1155
```

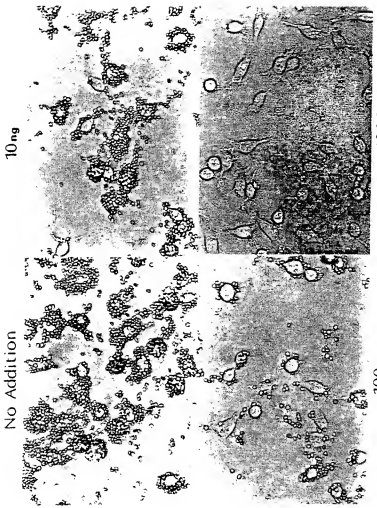
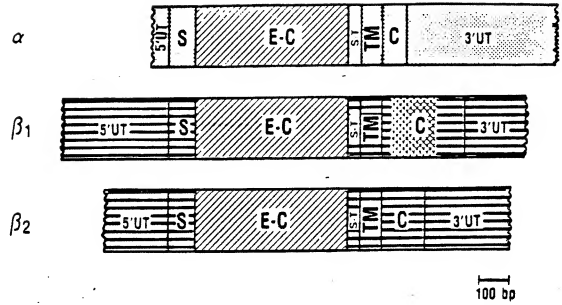


Figure 9

Figure 10


α
5'UT
S
E-C
I.S.
TM
C
3'UT
β1
5'UT
S
E-C
I.S.
TM
C
3'UT
β2
5'UT
S
E-C
I.S.
TM
C
3'UT
100 bp

INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/02845

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [2]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C12P 21/00; C12P 21/02; C12P 19/34; See Attachment
US CL : 435/68; 435/70; 435/91; 435/172.3; See Attachment

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/68,70,91,172.3,240.2,240.241,320 536/27 935/12,32,34,78

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

CHEMICAL ABSTRACTS DATA BASE (CAS) 1967-1988; BIOLOGICAL ABSTRACTS DATA BASE (BIOSIS) 1967-1988 KEYWORDS: IMMUNOGLOBULIN, Fe, HEAVY CHAIR, RECEPTOR, RECOMBINANT, PLASMID

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X,P	SCIENCE, (Washington, D.C.) Volume 234, issued 7 November 1986 (RAVETCH ET AL) "Structural Heterogeneity and Functional Domains of Murine Immunoglobulin G Fc Receptors". See pages 718-725..	1-25
X Y	US, A, 4,617,266 (FAHNESTOCK) 14 October 1986. See columns 5-8.	1-6,10-14,19 7-9,15-18, 20-25
X Y	JOURNAL OF BACTERIOLOGY (Washington, D.C.), Volume 167, issued 2 September 1986 (FAHNESTOCK ET AL) "Gene for an Immunoglobulin-Binding Protein from a Group G Streptococcus". See pages 870-880.	1-6,10-14, 19 7-9,15-18, 20-25

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
29 January 1988	10 FEB 1988
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Thomas D. Mays

Form PCT/ISA/210 (second sheet) (May 1986)

PCT/US87/02845

Attachment To Form PCT/ISA/210, Part I.

IPC(4): C12N 15/00; C12N 5/00; C07H 15/12; C07H 13/00

US CL : 435/240.2, 240.241; 536/27; 530/388

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No **
Y	PROCEDDINGS OF THE NATIONAL ACADEMY OF SCIENCES (USA) (Washington, D.C.), Volume 83, issued September 1986 (HIBBS ET AL), "The Murine Fc Receptor For Immunoglobulin: Purification, Partial Amino Acid Sequence, and Isolation of cDNA Clones". See pages 6980-6984.	1-25
X Y	THE EMBO JOURNAL (Oxford, UK), Volume 5, issued July 1986 (GUSS ET AL) "Structure of the IgG-Binding Regions of Streptococcal Protein G". See pages 1567-1575.	1-6,10-14, 19 7-9,15-18, 20-25
Y	NATURE (London) Volume 308, issued 1 March 1984 (MOSTOV ET AL) "The Receptor for Transepithelial Transport of IgA and IgM Contains Multiple Immunoglobulin-like Domains". See pages 37-43.	1-25